PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 05 September 2000 (05.09.00)	
International application No. PCT/EP00/01549	**Applicant's or agent's file reference** BET 00/0069
International filing date (day/month/year) 17 February 2000 (17.02.00)	**Priority date** (day/month/year) 19 February 1999 (19.02.99)
Applicant AMOUYEL, Philippe et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

31 July 2000 (31.07.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer F. Baechler
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
BET 00/0069		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/01549	17/02/2000	19/02/1999
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant INSTITUT PASTEUR DE LILLE		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
31/07/2000	15.02.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Jacques, P Telephone No. +49 89 2399 8934

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/01549

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-12 as originally filed

Claims, No.:

1-12 as originally filed

Drawings, sheets:

1/1 as originally filed

Sequence listing part of the description, pages:

1-2, filed with the demand

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 1-9 (with regard to industrial applicability).

because:

   ☒ the said international application, or the said claims Nos. 1-9 relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-12
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-9
	No:	Claims	10-12
Industrial applicability (IA)	Yes:	Claims	10-12
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. Claims 1- 9 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).
   In this context, the step a) of claim 1 is considered to fall under the concept of methods of surgery of the human/animal body (see further point 7 under Item V).

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Reference is made to the following document:

   D1: HANSEN ET AL.: 'Decreased tolbutamide-stimulated insulin secretion in healthy subjects with sequence variants in the high-affinity sulfonylurea receptor gene' DIABETES, vol. 47, no. 4, April 1998 (1998-04), pages 598-605.

2. As the particular combination of features of independent claim 1 is not disclosed in any cited prior art, the subject-matter of the said claim would appear to be novel (Article 33(2) PCT).

3. Moreover, the subject-matter of the said claim involves an inventive step in the sense of Article 33(3) PCT for the following reasons:
   The closest state of the art is considered to result from document D1.
   This document discloses that a silent polymorphism in exon 18 of the high-affinity *SUR1* gene is significantly associated with NIDDM (page 602, right column, lines 13-14). Moreover, the said variant in exon 18, in combination with an intron variant at

position -3 of the exon 16 (wherein "c" is replaced by "t") is also significantly associated with NIDDM (page 602, right column, lines 22-25). The said combination showing a reduced susceptibility towards sulfonylurea.

The subject-matter of claim 1 is distinguished therefrom in that a mutation in position -3 of intron 16 (namely in position-3 of the exon 17) (wherein "t" is replaced by "c") is shown to be associated with NIDDM.
The technical effect of this mutation is to confer higher susceptibility toward sulfonylurea.
The technical problem to be solved by the invention was therefore to provide a method for determining the suceptibility of a NIDDM patient toward sulfonylurea therapy.

The solution to the above mentioned technical problem has convincingly been solved by the identification of the association of the mutation in position -3 of the exon 17 of the *SUR1* gene with NIDMM and its involvement in suceptibility towards sulfonylurea. As the cited prior art does not give any hint of a correlation between the -3c allele of the *SUR1* gene and suceptibility toward sulfonylurea therapy, the subject-matter of claim 1 involves an inventive step in the sense of Article 33(3) PCT.

The same applies to dependent claims 2 to 9.

4. Notwithstanding the objection raised under Article 6 PCT (see point 1 under Item VIII), as the particular combination of features of independent claim 10 is not disclosed in any cited prior art, the subject-matter of the said claim would appear to be novel (Article 33(2) PCT).

5. However, the subject-matter of the said claim does not involve an inventive step in the sense of Article 33(3) PCT for the following reason:
document D1 discloses oligonucleotide sequences for PCR amplification of the 38 SUR1 exons and intron-exon boundaries (see page 599, right column, lines 28-32 and table 1)
It is thus considered that it would fall within the normal design capabilities of the skilled man to put a pair of the said oligonucleotides together in a kit, the said kit being suitable for amplifying all or part of the SUR1 gene comprising nucleotide -3

of intron 16.
Therefore the subject-matter of claim 10 does not meet the requirements of Article 33(3) PCT.

6. Dependent claims 11 and 12 do not appear to contain any additional features which meet the requirements of inventive steps as document D1 discloses the Pst I enzyme that specifically cuts fragments comprising nucleotide -3c/ nucleotide -3t (page 601, left column, line 11) and reagents to detect the presence of a cleaved fragment (see page 600, the "Detection of sequence variants in the SUR1 gene" section).

7. For the assessment of the present claims 1-9 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VIII
Certain observations on the international application

1. The subject-matter of claim 10 is not clear (Art. 6 PCT) for the following reason:
the expression "instructions relating to detecting the presence of a -3c allele of intron 16 and correlating the presence of a -3c allele with a higher susceptibility toward sulfonylurea therapy" is considered as meaning directions on how to perform a mental or physical act. As such it may be equated to a process. Thus, these features in the product claim relate to a method of using the product rather than clearly defining the product in terms of its technical features. The intended limitations are therefore not clear from this claim, contrary to the requirements of Article 6 PCT.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/01549

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
STRAND, EPO-Internal, WPI Data, PAJ, MEDLINE, EMBASE, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HANSEN ET AL.: "Decreased tolbutamide-stimulated insulin secretion in healthy subjects with sequence variants in the high-affinity sulfonylurea receptor gene" DIABETES, vol. 47, no. 4, April 1998 (1998-04), pages 598-605, XP002109828 cited in the application the whole document	1-12
A	WO 97 18308 A (SAKURA HIROSHI ;ASHCROFT FRANCES (GB); ASHCROFT STEPHEN JOHN HASLA) 22 May 1997 (1997-05-22) page 22-23; claim 23	1-12

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 July 2000	01/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Reuter, U

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EE	Estonia	**LR**	Liberia	**SG**	Singapore		

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/01549

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ASHCROFT ET AL.: "Mechanisms of the glycaemic effects of sulfonylureas" HORMONE AND METABOLIC RESEARCH, vol. 28, no. 9, 1996, pages 456-463, XP002109829 the whole document	1-12
A	WO 95 28411 A (BAYLOR COLLEGE MEDICINE ;UNIV TEXAS (US)) 26 October 1995 (1995-10-26) page 3-5 page 47-53; claims	1-12
A	WO 98 14571 A (INCYTE PHARMA INC ;COLEMAN ROGER (US); AU YOUNG JANICE (US); BANDM) 9 April 1998 (1998-04-09) page 1-2 page 23-34	1-12

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/01549

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9718308 A	22-05-1997	AU 7583296 A	05-06-1997
WO 9528411 A	26-10-1995	US 5863724 A	26-01-1999
		AU 694507 B	23-07-1998
		AU 2285595 A	10-11-1995
		CA 2187945 A	26-10-1995
		EP 0789705 A	20-08-1997
		JP 9512166 T	09-12-1997
		US 6031150 A	29-02-2000
		US 6054313 A	25-04-2000
WO 9814571 A	09-04-1998	AU 4742297 A	24-04-1998
		EP 0932672 A	04-08-1999

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12Q 1/68

A1

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(43) International Publication Date: 24 August 2000 (24.08.00)

(21) International Application Number: PCT/EP00/01549

(22) International Filing Date: 17 February 2000 (17.02.00)

(30) Priority Data:
99400410.9 19 February 1999 (19.02.99) EP

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(72) Inventors; and

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(74) Agent: JACOBSON, Claude; Cabinet Lavoix, 2, place d'Estienne d'Orves, F–75441 Paris Cedex 09 (FR).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHOD FOR DETERMINING THE SUSCEPTIBILITY OF A NIDDM PATIENT TOWARD SULFONYLUREA THERAPY

(57) Abstract

This invention concerns a method for determining the susceptibility of a NIDDM patient toward sulfonylurea therapy comprising: a) obtaining a sample from a NIDDM patient, said sample comprising nucleic acid molecules containing the fragment of the *SUR1* gene comprising the nucleotide in position –3 of intron 16; b) detecting the presence or the absence of the –3c allele in position –3 of intron 16, whereby the presence of at least one –3c allele identifies a NIDDM patient with a higher susceptibility toward sulfonylurea therapy.

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:downstream primer

<400> 3
ggaggatggt taaaaggaga tt 22

1

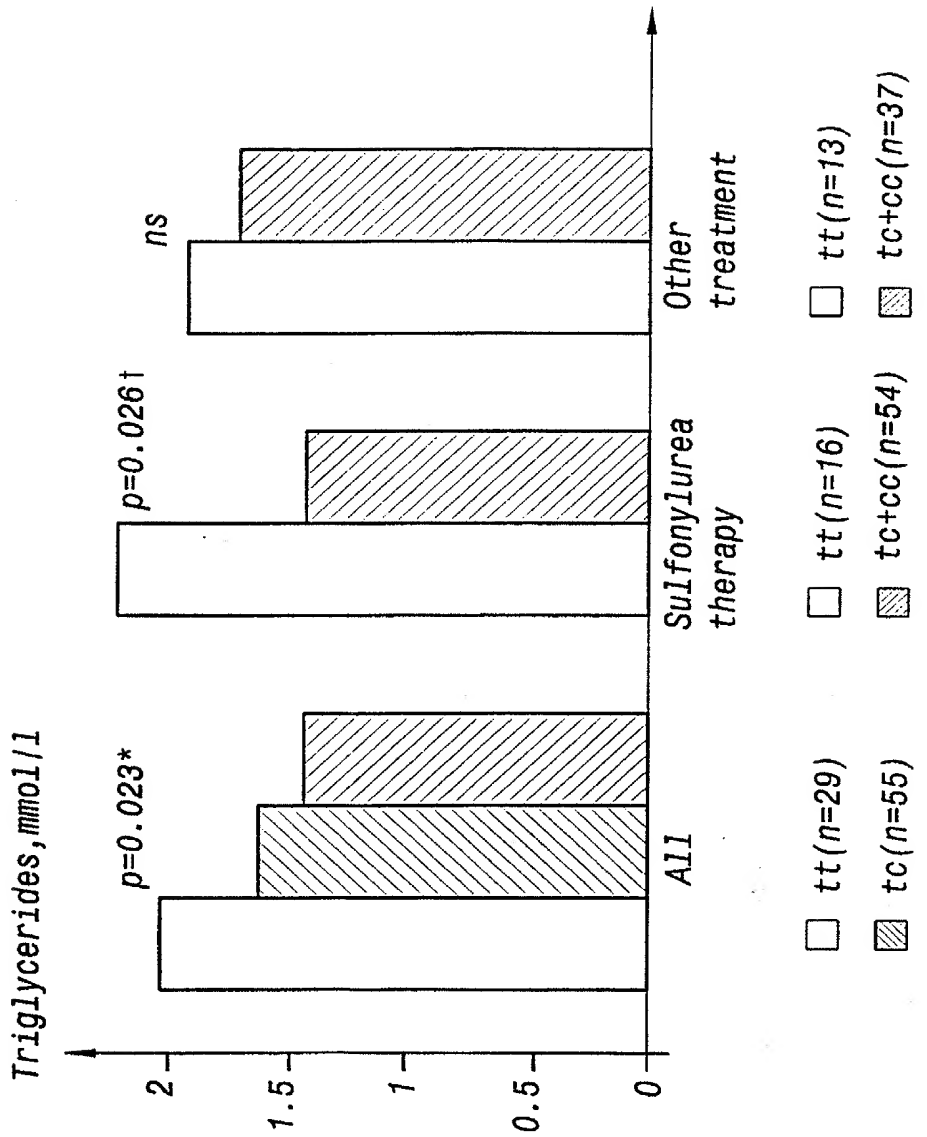
2

1

2

1

2


Triglycerides, mmol/l
2
1.5
1
0.5
0
p=0.023*
p=0.026†
ns
All
Sulfonylurea therapy
Other treatment
tt (n=29)
tc (n=55)
cc (n=36)
tt (n=16)
tc+cc (n=54)
tt (n=13)
tc+cc (n=37)

Method for determining the susceptibility of a NIDDM patient toward sulfonylurea therapy.

The present invention relates to a method of determining the susceptibility of a non-insulin-dependent diabetes mellitus (NIDDM) patient toward a sulfonylurea therapy.

Sulfonylureas are oral hypoglycaemic agents widely used in the treatment of NIDDM. They bind to the high affinity sulfonylurea receptor 1 (SUR1) and stimulate insulin release from pancreatic islet β cells. SUR1 is one of the protein that composes the ATP-sensitive potassium channel I_{KATP}, closed by glucose metabolism in pancreatic β cells and triggering insulin exocytosis. The gene encoding SUR1 is located on chromosome 11p15.1. Mutations in the gene have been found in Familial Persistent Hyperinsulinemic Hypoglycaemia of Infancy (PHHI) (Thomas et al, (1995) ; Thomas et al (1986), Kane et al (1996) and Dunne et al (1997)) also known as Familial Hypersinsulinism (HI) (Nestorowicz et al (1996)). This disease is characterized by the elevation of serum insulin levels and severe hypoglycaemia.

Two case-control studies reported an association between genetic polymorphisms in the *SUR1* gene and NIDDM. (Inoue et al, (1996) Hansen et al (1998)). To estimate the impact of the *SUR1* genetic variability on NIDDM in population, the inventors characterized the genotypes of subjects for the most frequent polymorphism of the *SUR1* gene, a –3t→c mutation located in intron 16, namely in position –3 of the exon 17 splice acceptor site (nucleotide 191 of SEQ ID n° 1) in a large representative sample of the French population aged 35 to 64 years.

As a result, they discovered that among the NIDDM patients, the frequency of the c allele was significantly lower in controls than in NIDDM patients.

In controls, no association was found between the polymorphism and body mass index, waist-to-hip ratio, fasting plasma glucose, fasting plasma insulin and lipid and lipoproteins profile. In NIDDM patients, the c allele was associated with a decrease in plasma triglycerides concentrations. NIDDM patients were stratified in two groups : subjects treated with sulfonylureas and subjects treated without. Decreases in plasma triglycerides and VLDL-cholesterol concentrations were found only in c allele bearers treated with sulfonylureas.

The discovery that sulfonylurea therapy seems to be more efficient on hypertriglyceridemia reduction in NIDDM patients with the *SUR1* intron 16 c allele than in NIDDM patients without, may help to a better targeting of the various therapies available in NIDDM treatment.

The present invention relates to a method for determining the susceptibility of a NIDDM patient toward sulfonylurea therapy comprising :

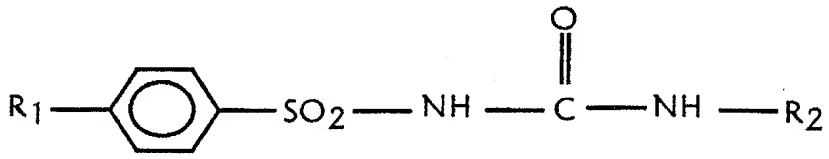
a) obtaining a sample from a NIDDM patient, said sample comprising nucleic acid molecules containing the fragment of the *SUR1* gene comprising the nucleotide in position –3 of intron 16,

b) detecting the presence or the absence of the –3c allele in position –3 of intron 16,

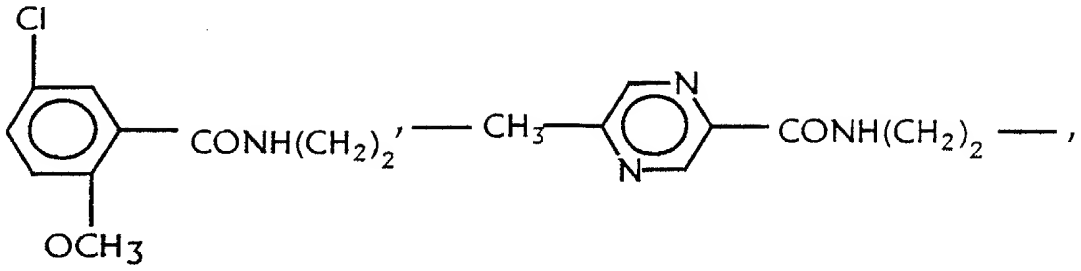
whereby the presence of at least one –3c allele identifies a NIDDM patient with a higher susceptibility toward sulfonylurea therapy.

Sulfonylurea therapy in the sense of the instant invention identifies the current therapies of NIDDM utilizing oral hypoglycaemic agents binding the SUR1 receptor and stimulating insuline release from pancreatic islet β cells.

Such agents are derivatives of arylsulfonylurea having the following general formula :



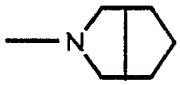
wherein R_1 may have the following meanings : Cl, CH_3,

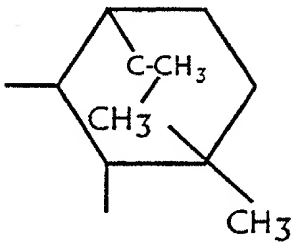


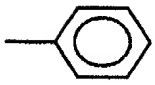
and R_2 may have the following meanings :

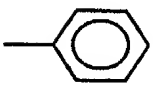
$-(CH_2)_2CH_3$

$-(CH_2)_3CH_3$









The main compounds are known under the following denominations : chlorpropamide, tolbutamide, gliclazide, glibornuride, glibenclamide, glipizide and buformine.

The sample from the patient may be any biological sample containing nucleic acids, namely a blood sample.

Intron 16 of the *SUR1* gene is identified within the instant invention according to the nomenclature of Hansen et al (above) which teaching is hereby incorporated by reference.

The mutation responsible for the polymorphism referred to in the instant invention occurs on nucleotide -3 of the exon 17 splice acceptor site, the first nucleotide of the intron being numbered –1, the second –2 and the third –3 (SEQ ID n° 1, EMBL accession number L78223).

The detection of the –3t→c mutation in intron 16 may be performed by any known method in the art detecting DNA sequence variation.

A review of currently available methods of detecting DNA sequence variation can be found in a review by Grompe (1993).

In a preferred embodiment, the method comprises prior to step b) the step of amplifying said nucleic acid molecules using amplification primers that selectively anneal to and amplify a portion of said gene comprising the nucleotide in position –3 of intron 16.

One method for detecting the –3t→c mutation in the position –3 of intron 16 of the *SUR1* gene comprises sequencing all or part of the sequence of intron 16 comprising said –3 nucleotide.

Direct DNA sequencing, either manual sequencing or automated fluorescent sequencing may be used.

Another approach is the single-stranded conformation polymorphism assay (SSCA') (Orita et al, 1989).

According to that approach, step b) comprises obtaining a first *SUR1* gene fragment comprising the nucleotide in position –3 of intron 16 isolated from a human sample and a second *SUR1* gene fragment comprising nucleotide –3t of intron 16, said second fragment corresponding to said first fragment, forming single-stranded DNA from said first *SUR1* gene fragment and from said second *SUR1* gene fragment, electrophoresing said single-stranded DNAs on a denaturating polyacrylamide gel, comparing the mobility of said single-stranded DNAs on said gel to determine if said single-stranded DNA from said first *SUR1* gene fragment is shifted relative to said second *SUR1* gene fragment.

The fragments which have shifted mobility on SSCA gels are then optionally sequenced to determine the exact nature of the DNA sequence variation.

Alternatively, instead of utilizing as the second gene fragment a gene fragment comprising nucleotide –3t of intron 16, one may utilize a second fragment comprising nucleotide –3c of intron 16, whereby similar mobility is indicative of the presence of the –3t→c mutation in the position –3 of intron 16.

An other approach comprises contacting the nucleic acid molecules with a nucleic acid probe that selectively hybridizes to a portion of said 16 intron of *SUR1* gene containing nucleotide –3 as shown in sequence SEQ ID n° 1 under hybridization conditions.

A further method comprises performing a restriction endonuclease digestion of said nucleic acid molecules thereby yielding a nucleic acid digest and contacting the digest with a nucleic acid probe that selectively hybridizes to a portion of said intron 16 of said *SUR 1* gene containing nucleotide –3 as showed in sequence SEQ ID n° 1.

A still a further method comprises amplifying all or part of the *SUR1* gene in said sample using a primer specific for allele –3c and detecting the presence of an amplified product, whereby the presence of said product indicates the presence of said allele in the sample.

By "higher susceptibility", it is intented that not only hyperglycemia is decreased in NIDDM patients, but also hypertriglyceridemia, which is the main factor of cardiac risk for diabetic patients.

The instant invention also relates to a kit for determining the susceptibility of a NIDDM patient toward sulfonylurea therapy comprising a pair of nucleotide primers specific for amplifying all or part of the *SUR1* gene comprising the nucleotide –3 of intron 16, and instructions relating to detecting the presence or a –3c allele of intron 16 and correlating the presence of a –3c allele with a higher susceptibility toward sulfonylurea therapy.

In a preferred embodiment, the kit comprises a restriction enzyme that specifically cuts fragments comprising nucleotide –3c/nucleotide –3t, and reagents able to detect the presence of a cleaved fragment, the presence of a

cleaved fragment being indicative of a higher susceptibility toward sulfonylurea therapy (-3c)/ a lower susceptibility toward sulfonylurea therapy (-3t).

The restriction enzyme is preferably *Pst I* that cleaves specifically fragments comprising the –3c allele.

The present invention is described by reference to the following examples and the enclosed figure representing the effect of the *SUR 1* intron 16 –3t→c polymorphism on plasma triglycerides concentrations in NIDDM subjects, wherein * indicates that the p value was adjusted for age, gender, body mass index, alcohol and smoking consumptions (linear trend test) and † is indicative that the test used was the non-parametric Wilcoxon test.

Standard techniques well known in the art or the techniques specifically described below were utilized.

Population and methods

Population study

The population study was selected in 1995-1997 from three large representative French samples participating to the risk factor surveys of the WHO-MONICA (Multinational Monitoring of trends and determinants of Cardiovascular diseases) project (Ecological analysis of the association between mortality and major risk factors of cardiovascular disease. The World Health Organization MONICA Project. Int J. Epidemiol. 1994 : 23:505-16 ; Tunstall-Pedoe H et al). This population study was randomly sampled from the electoral rolls of three geographical areas : the Urban Community of Lille (Lille) in the North, the department of Bas-Rhin (Strasbourg) in the East, the department of Haute-Garonne (Toulouse) in the South of France. The number of subjects recruited were 1195, 1131 and 1182 in Lille, Strasbourg and Toulouse areas respectively stratified on ten year age classes and gender. A fasting blood sample was drawn for all participants. In these samples, 123 NIDDM affected individuals were recovered on the basis of a medical diagnosis and on the existence of a specific treatment (Lille n=47, Strasbourg n=41, Toulouse n=35). A control group (n=1250) composed of individuals without

diabetes, hypercholesterolemia or hypertension and without any treatment for these diseases was selected.

Biological measurements

Glucose was measured by the glucose oxidase method (DuPont Dimension). Plasma insulin was measured by radio-immunoassay (BiInsuline, ERIA Pasteur). Serum total cholesterol and triglyceride levels were measured by enzymatic methods (DuPont Dimension).

Genetic analysis

Genomic DNA was extracted from white blood cells as described by Miller, A et al (1988). DNA amplification was performed using Polymerase Chain Reaction (PCR). Typing of the intron 16 –3t→c polymorphism was achieved as described by Inoue et al (above).

Statistics

Statistical analyses were performed with the SAS statistical software, version 6.11 (SAS Institute Inc., Cary, NC). Genotype and allele distributions were compared with Pearson λ^2 statistical tests. The effect of the polymorphism on quantitative variables was tested with a multivariate analysis of covariance using a general linear model (proc GLM, type III SS). Interactions between genotypes and covariates were tested. Data for triglycerides, insulin, and glucose were log transformed to normalize the distributions. Statistical significance was considered at the $p<0.05$ level. When the number of subjects was low, genotypes were compared using non parametric Wilcoxon test.

Results

In control subjects, the frequency of the c allele of the *SUR* intron 16 –3t→c polymorphism was 0.45, 0.44 and 0.50 in Lille, Strasbourg and Toulouse studies respectively. When controls of the three studies were pooled, the frequency of the c allele was 0.46 while it was 0.53 in NIDDM patients. The

adjusted relative risk for *cc* subjects to develop NIDDM was 1.76 (95 % CI : [1.10-2.80], p=0.017) adjusted for age, gender, centre and body mass index.

Possible associations between the intron 16 –3t→c polymorphism and clinical and biological variables such as body mass index (BMI), waist-to-hip ratio, plasma insulin, fasting-plasma glucose, or lipid variables in control and in NIDDM subjects were investigated. In controls, no association was found between the polymorphism and any variables listed above. In NIDDM patients, the -3c allele was associated with decreased plasma triglycerides concentrations (2.03 [1.12-3.71] for *tt*, 1.62 [0.88-2.97] for *tc* and 1.45 [0.85-2.46] mmol/l for *cc*, p=0.023) and in an allele dose dependent manner (Table 2, figure 1).

As the sulfonylurea receptor 1 binds sulfonylurea agents, NIDDM patients were stratified in two groups : NIDDM patients receiving sulfonylureas (n=70) and patients receiving another treatment (n=52). Given the low number of *cc* homozygous subjects, *tc* and *cc* subjects were pooled to analyse a dominant effect of the –3c allele. Wilcoxon tests were performed. In the group treated with sulfonylureas, the intron 16 –3c allele was associated with a statistically significant decrease in plasma triglycerides concentrations (2.20 mmol/l [1.14-4.14] for *tt* versus 1.43 mmol/l [0.81-2.52] for *tc*+ *cc* ; p=0.026) whereas no association was found in the other group (figure 1).

The results indicate that the -3c allele is associated with decreased plasma triglycerides concentrations in NIDDM patients, only in NIDDM patients receiving a sulfonylurea therapy, underlying a pharmacogenetic susceptibility to sulfonylurea treatment response.

This result is in accordance with previous works showing that oral sulfonylurea therapy, in addition to an improvement of glycemic control, decreases hepatic lipase levels and declines the production of triglycerides and VLDL-cholesterol in diabetics (Howard et al (1985) ; Taskinen et al (1986)). The results of the instant invention suggest that sulfonylurea therapy is more efficient on hypertriglyceridemia reduction in NIDDM patients bearing the *SUR1* intron 16 –3c allele underlying a pharmacogenetic susceptibility to sulfonylurea treatment response.

Table 1 : Genotype and allele frequencies of the *SUR* intron 16 3t→c polymorphism in NIDDM patients and control subjects.

	NIDDM patients				Controls
	Lille	Strasbourg	Toulouse	All	
n	47	41	34	122	1250
Genotype frequencies					
tt	9 (0.19)	11 (0.27)	9 (0.27)	29 (0.24)	359 (0.29)
tc	24 (0.51)	18 (0.44)	14 (0.41)	56 (0.46)	620 (0.50)
cc	14 (0.30)	12 (0.29)	11 (0.32)	37 (0.30)*	271 (0.21)
allele frequencies					
t	42 (0.45)	40 (0.49)	32 (0.47)	114 (0.47)	1338 (0.54)
c	52 (0.55)	42 (0.51)	36 (0.53)	130 (0.53)†	1162 (0.46)

Data are n(frequency %). Controls include Lille, Strasbourg, Toulouse studies.

* All NIDDM subjects versus controls *cc/tc+ tt*, p= 0.03.

† All NIDDM subjects versus controls *c/t*, p=0.04.

Table 2 : Effect of the *SUR 1* intron 16-3 t→c polymorphism in NIDDM subjects.

Genotype intron 16	*tt*	*tc*	*cc*	*p*
n	29	55	36	
BMI, kg/m^2	30.7±5.2	29.9±6.2	30.1±6.2	ns
Insulin, µU/ml	20.25 [11.73-34.95]	17.64 [10.28-30.26]	16.78 [8.50-33.11]	ns
Glucose, mmol/l	8.25 [6.05-11.25]	8.25 [6.05-11.25]	8.67 [6.49-11.59]	ns
total cholesterol, mmol/l	5.89±0.94	5.69±0.97	5.61±1.28	ns
Triglycerides, mmol/l	2.03 [1.12-3.71]	1.62 [0.88-2.97]	1.45 [0.85-2.46]	0.023*

* p value was adjusted for age, gender, BMI, alcohol consumption and smoking consumptions (test for linear trend)

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CLAIMS

1. A method for determining the susceptibility of a NIDDM patient toward sulfonylurea therapy comprising :

a) obtaining a sample from a NIDDM patient, said sample comprising nucleic acid molecules containing the fragment of the *SUR1* gene comprising the nucleotide in position –3 of intron 16,

b) detecting the presence or the absence of the –3c allele of intron 16,

whereby the presence of at least one –3c allele identifies a NIDDM patient with a higher susceptibility toward sulfonylurea therapy.

2. A method according to claim 1, further comprising prior to step b) the step of amplifying said nucleic acid molecules using amplification primers that selectively anneal to and amplify a portion of said gene comprising the nucleotide in position –3 of intron 16.

3. A method according to claim 2 wherein said amplification primers are the nucleic acid fragments of sequence SEQ ID N° 2 and SEQ ID n° 3.

4. A method of claim 1, wherein said detecting step b) comprises sequencing all or part of the sequence of intron 16 comprising said –3 nucleotide.

5. The method of claim 1, wherein said detecting step b) comprises contacting the nucleic acid molecules with a nucleic acid probe that selectively hybridizes to a portion of said 16 intron of *SUR1* gene containing nucleotide -3 as shown in sequence SEQ ID n° 1 under hybridization conditions.

6. The method of claim 1, wherein the detecting step b) comprises performing a restriction endonuclease digestion of said nucleic acid molecules thereby yielding a nucleic acid digest and contacting the digest with a nucleic acid probe that selectively hybridizes to a portion of said intron 16 of said *SUR 1* gene combining nucleotide –3 as showed in sequence SEQ ID n° 1.

7. The method of claim 1, wherein said detecting step b) comprises obtaining a first gene fragment comprising nucleotide –3 of intron 16

isolated from said human sample and a second gene fragment comprising nucleotide –3t of intron 16, said second fragment corresponding to said first fragment, forming single-stranded DNA from said *SUR1* gene fragment and from said second *SUR1* gene fragment, electrophoresing said single-stranded DNAs on a denaturating polyacrylamide gel, comparing the mobility of said single-stranded DNAs on said gel to determine if said single-stranded DNA from said first *SUR1* gene fragment is shifted relative to said second *SUR1* gene fragment, and optionally sequencing said single-stranded DNA from said first *SUR1* gene fragment having a shift in mobility.

8. The method of claim 1 wherein said detecting step b) comprises obtaining a first gene fragment comprising nucleotide –3 of intron 16, isolated from said human sample and a second fragment comprising nucleotide –3c of intron 16, said second fragment corresponding to said first fragment, forming single-stranded DNA from said *SUR1* gene fragment and from said second *SUR1* gene fragment, electrophoresing said single-stranded DNAs on a denaturating polyacrylamide gel, comparing the mobility of said single-stranded DNAs on said gel to determine if said single-stranded DNA from said first *SUR1* gene fragment has the same mobility as the said second *SUR1* gene fragment, and optionally sequencing said single-stranded DNA from said first *SUR1* gene fragment.

9. The method of claim 1 wherein said detecting step b) comprises amplifying all or part of a *SUR1* gene in said sample using a primer specific for allele –3c and detecting the presence of an amplified product, whereby the presence of said product indicates the presence of said allele in the sample.

10. A kit for determining the susceptibility of a NIDDM patient toward sulfonylurea therapy comprising a pair of oligonucleotide primers specific for amplifying all or part of the *SUR1* gene comprising nucleotide –3 of intron 16, and instructions relating to detecting the presence of a –3c allele of intron 16 and correlating the presence of a –3c allele with a higher susceptibility toward sulfonylurea therapy.

11. A kit according to claim 10 comprising a restriction enzyme that specifically cuts fragments comprising nucleotide –3c/nucleotide –3t, and

reagents able to detect the presence of a cleaved fragment, the presence of a cleaved fragment being indicative of a higher susceptibility toward sulfonylurea therapy (-3c)/a lower susceptibility toward sulfonylurea therapy (-3t).

12. A kit according to claim 11 wherein said enzyme is *Pst I*.

Add A9

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INSERM

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